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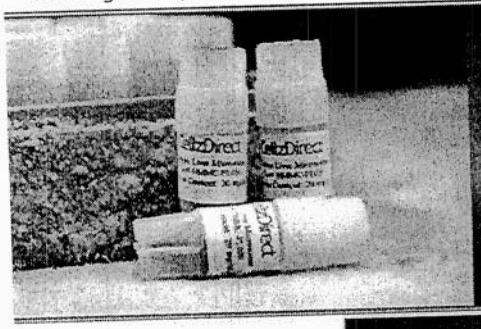

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Product Applications

CellzDirect's experienced technical support and scientific teams would be happy to discuss all of the applications for which our products may be used. Below is a quick reference guide that might help to determine the most appropriate product for your application. We would be happy to help match your specific need to a product type or product lot that we have available.

Drug Metabolism Product Applications

Application	Microsomes	S9 Fractions	Fresh Hepatocytes	Cryopreserved Hepatocytes
Inhibition	✓		✓	✓
Reaction Phenotyping	✓			
Metabolic Stability	✓	✓	✓	✓
Intrinsic Clearance	✓		✓	✓
Metabolic Profiling /Met ID	✓	✓	✓	✓
Induction			✓	✓

Transporter Product Applications

Application	Transporter Membranes	Transporter Vesicles	Fresh Hepatocytes	Cryopreserved Hepatocytes
Inhibition	✓	✓	✓	✓
Substrate Potential	✓	✓	✓	✓
Efflux	✓	✓	✓	✓
Uptake			✓	✓
Induction			✓	✓

Product Background

The primary goal of pharmaceutical companies is to discover and develop safe and effective drugs. The drug discovery process is costly and time-consuming, making drug discovery and development a financially risky business. It has been estimated that it requires hundreds of millions of dollars and at least 10 years for a single new chemical entity (NCE) to reach the marketplace as a new drug. In order for a NCE to make it to market as a safe and effective drug, it must pass through a series of hurdles. The first hurdle to overcome is passing from drug discovery to the preclinical phase. The preclinical phase evaluates initial parameters such as: drug metabolism, pharmacokinetics, and toxicity in animals. After successfully passing through the preclinical stage the NCE is then submitted

RapidAlert »

as an investigational new drug (IND) application to the Food and Drug Administration (FDA). If approved, then the IND may enter the full development process: Phase I (safety and tolerability in healthy volunteers); Phase II (efficacy in a small number of patients); and Phase III (efficacy in a large population of patients). If the drug passes all three clinical (human) phases, it is submitted to the FDA as a new drug application (NDA). Upon FDA approval, the drug then enters the market place. It is also worth noting that approximately 1 in 4 marketed drugs generate sufficient revenue to recover the costs associated with its discovery and development.

A significant factor that increases costs in the drug development process is the high failure rates of NCEs resulting in many false starts or unnecessary development costs. It has been estimated that 1 in 5000 compounds make it from drug discovery to the preclinical stage. In addition, only 1 in 25 compounds that make it from the preclinical stage to the IND stage will make it to market as a new drug.

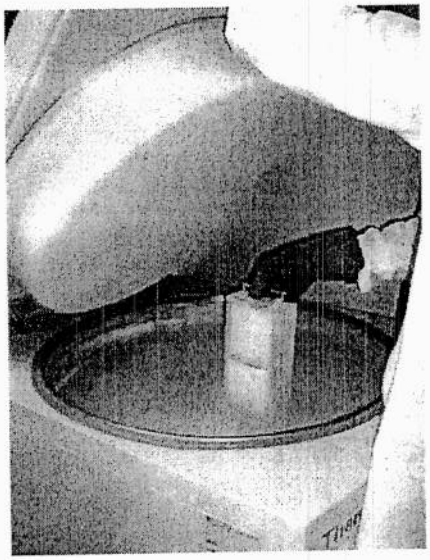
The ever-increasing number of NCEs synthesized by pharmaceutical companies has prompted the development of research methods for rapid screening. Present research efforts are directed at developing a series of in vitro assays that could serve as reliable indicators to minimize the attrition rates of new chemical entities. By utilizing in vitro models, pharmaceutical companies are now focused on reducing these preclinical and clinical failure rates by attempting to accurately evaluate efficacy and safety much earlier in the drug discovery process. Ideal in vitro models to predict drug metabolism are derived from human liver tissues such as intact liver cells (hepatocytes) and subcellular fractions of the hepatic smooth endoplasmic reticulum (microsomes).

Microsomes and hepatocytes are effective *in vitro* models that are used as biological tools for obtaining information about the metabolism of NCEs as they move through the drug development process. The majority of drug metabolism occurs in the liver by cytochrome P450 enzymes (CYP). These enzymes are primarily located in subcellular organelles (the smooth endoplasmic reticulum). Presently, five major CYPs have been identified to be involved in the vast majority of all marketed drugs: CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Significant factors in eliminating NCEs as potential drugs are CYP dependent drug-drug interactions and rapid CYP dependent metabolism of a drug (metabolic stability). CYP-related drug-drug interactions occur either when one drug inhibits or increases the metabolism of another co-administered drug. For example, the FDA recall of the first non-sedating antihistamine (Seldane) was based on it's potential to reach lethal blood levels when co-administered with an antibiotic such as erythromycin (1997). In this case, both drugs are primarily metabolized by CYP3A4, and subsequently when co-administered, their elimination phases are hindered. The clinical result was an increase in the blood levels of Seldane to toxic levels resulting in lethal arrythmias. Microsomes and hepatocytes are effective *in vitro* models that can be used to predict drug-drug interactions as well as metabolic stability. Pharmaceutical companies routinely use microsomes and hepatocytes in the drug discovery process in attempts to reduce the attrition rates of NCEs and the sky rocketing costs of the drug development process.

Prior to the availability of human tissues for medical research, scientists had to rely on data obtained from animals and animal tissues. Unfortunately, animal studies do not adequately predict what will happen in humans because of the basic physiological differences between each species. With the advent of obtaining consent of human tissues to be used in medical research, scientists can better predict how a new drug will fare in humans. It cannot be overstated that the availability of human tissue is crucial to developing more safe and effective drugs.

CellzDirect provides fresh human hepatocytes on a frequent basis as suspensions and in multi-well plates. For notification of fresh hepatocyte availability, please register for our RapidAlert service. Cryopreserved human suspensions are also available from a wide range of donors. Fresh animal hepatocytes are provided on a scheduled monthly basis as suspensions or in multi-well plates. All species and strains of animal toxicology models are available as cryopreserved suspensions. Custom isolations are provided upon request. Human and animal subcellular fractions, including liver microsomes, S9, and cytosol, are also available. CellzDirect also offers a comprehensive selection of ATP-binding cassette (ABC) transporters, through our partnership with GenoMembrane.

Our experienced scientific staff conducts ongoing research with hepatocyte culture systems to remove technological barriers to *in vitro* testing, improve cell quality and extend their utility. With CellzDirect's support and cell products, scientists are better able to conduct research that accelerates the discovery of safe and effective drugs.



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May 25-27, 2009

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Organizing Chairs:
Albert P. Li, APSciences/In Vitro ADMET Laboratories, LLC
Rene H. Levy, University of Washington

Pre-conference Workshop-2009

Monday, May 25, 2009

Current Practice: Enzyme Induction and Uptake Transporter Assays
Chair: Albert P. Li

8:00 AM – 9:00 AM – REGISTRATION

8:50 AM – 9:00 AM - EXHIBITOR PRESENTATION

XENOTECH

Pre-Conference Session I: Enzyme Induction Assays

9:00 AM – 9:40 AM
Key Parameters for the Design of Enzyme Induction Studies *(Steve Ferguson, Invitrogen; Durham, NC)* Cultures of primary human hepatocytes are recognized as the "gold standard" for assessing induction potential *in vitro*. In this presentation we will outline important considerations in the design of in vitro induction studies using primary hepatocytes and review example data demonstrating the importance of healthy hepatocyte monolayers and need for sufficient endpoints (e.g. cell health, mRNA, protein) to effectively interpret and translate *in vitro* data to *in vivo* relevance. In addition, related endpoints (e.g. cytotoxicity, metabolism, transport etc…) will be discussed.

9:40 AM – 10:20 AM
Nuclear Receptors and Drug-Drug Interaction *(Jerome M. Lasker, Hackensack University Medical Center; Hackensack, NJ)* Over the past decade, orphan nuclear receptors (PXR, CAR and PPAR□) have been shown to play an integral role in the induction of liver and extrahepatic P450 enzymes by therapeutic agents. Drugs act as ligands for these receptors, which then translocate as a complex into the nucleus, and activate transcription of specific P450 genes. We will discuss here how such receptor-mediated P450 enzyme induction can lead to marked interactions between drugs administered concurrently.

10:20 AM – 10:50 AM – BREAK

10:50 AM – 11:00 AM - EXHIBITOR PRESENTATION

BRI Biopharmaceutical Research Inc.

11:00 AM – 11:40 AM

Evaluation of P450 Induction Using Hepatic Cell Lines *(Odette Fahmi, Pfizer; Groton, CT)* In recent years, various *in vitro* assays have been developed and used routinely to assess the potential for drug-drug interactions due to CYP induction. The experimental conditions, advantages and disadvantages of these systems will be reviewed.

11:40 AM – 12:20 PM
Higher Throughput Assay for P450 Induction *(Albert P. Li, APSciences/IVAL; Columbia, MD)* A higher throughput assay for P450 induction for CYP1A2 and CYP3A4 will be discussed. Plateable cryopreserved human hepatocytes cultured on 96- and 384-well plates are used for the assay. The P450 assays are ethoxyresorufin-O-deethylation and IPA-luciferin dealkylation, with activities quantified based on fluorescence and luminescence, respectively, using a multi-channel plate reader. Results with known inducers are shown.

12:20 PM – 2:00 PM – LUNCH BREAK

Pre-conference Session II: Uptake Transporter Assays

2:00 PM – 2:10 AM - EXHIBITOR PRESENTATION

APSciences, Inc. | IVAL In Vitro ADMET Laboratories, LLC

2:10 PM – 2:50 PM
Inhibition of Bile Acid transport and Determination of the Cholestatic Potential of Drug Candidates *(Kenneth R. Brouwer; Smith, William R.; Clark, Jennifer C.; St. Claire, Robert L.; Perry, Cassie H.; Ansede, John; Qualyst; Durham, NC)* Drug-induced cholestasis may result from the inhibition of biliary efflux of bile acids in the liver. Increased serum concentrations of bile acids can result from inhibition of uptake or efflux transporters, and may not be reflective of the intracellular concentration of bile acids which are responsible for hepatotoxicity. Sandwich-cultured hepatocytes (BCLEAR®) that maintain the expression, localization, and function of hepatic uptake and efflux transporters were used to evaluate and predict the potential of a compound to cause transporter based cholestatic hepatotoxicity. D_8-taurocholate was used as a probe in 24-well rat and human sandwich-cultured hepatocytes.

2:50 PM – 3:20 PM
Current Practice: Evaluation of Uptake Transporter Activities in Human Hepatocytes *(MingXiang Liao, Millennium Pharmaceutical, Inc.; Cambridge, MA)* Organic anion transporting polypeptides (OATPs), members of solute carrier class (SLC) superfamilies, are important uptake transporters involving in the absorption and elimination of a large variety of endogenous and exogenous substrates. Hepatic OATPs are widely recognized to play important roles in drug disposition and hepatotoxicity in liver. Primary hepatocytes are now recognized as one of the most relevant and practical models with which the study of drug metabolism and transporter interactions is best performed. In this presentation, the evaluation of the uptake transporter activities in hepatocytes will be reviewed along with the progress in the utilization of human hepatocytes in OATP-mediated transport studies in our group.

3:20 PM – 3:50 PM – BREAK

3:50 PM – 4:30 PM
Evaluation of Uptake Transporter Activities in Cultured Human Hepatocytes *(Pieter Annaert, Katholieke Universiteit Leuven; Leuven, Belgium)* Data will be presented to demonstrate the utility of sandwich-cultured hepatocytes as a unique in vitro tool for evaluating hepatobiliary drug disposition. In addition, the model allows in vitro assessment of possible drug-interactions mediated by hepatic uptake transporters. In this study, eight marketed HIV protease inhibitors are used as model interacting drugs.

4:30 PM - 5:00 PM – PANEL DISCUSSION

END OF DAY

END OF PRE-CONFERENCE

Main Conference
DDI-2009
12th International Conference on Drug-Drug Interactions

Tuesday, May 26, 2009

Session I: General Overview of DDI Status
Chair: René Levy

8:00 AM – 9:00 AM – REGISTRATION

8:50 AM – 9:00 AM - EXHIBITOR PRESENTATION

University of Washington
Metabolism and Transport
Drug Interaction Database

9:00 AM – 9:15 AM
OPENING REMARKS: Overview: Science of DDI – Where are We Today? *(René Levy, University of Washington, Seattle, WA)*

9:15 AM – 9:55 AM
NMEs Approved in 2008-9 *(Carol Collins, University of Washington, Seattle, WA)* This presentation will discuss information found in posted NDA reviews for drugs approved in 2008. Emphasis will be placed on FDA comments extracted from the NDAs that address issues not covered in the draft guidance, Drug Interaction Studies-Study Design, Data Analysis, and Implications for Dosing and Labeling.
(http://www.fda.gov/cder/guidance/6695dft.pdf).

9:55 AM – 10:35 AM
Critical Review of the Literature 2008-2009 *(Houda Hachad, University of Washington and Isabelle Ragueneau-Majlessi, University of Washington; Seattle, WA)* This presentation will provide an update on the literature on drug interactions in 2008-2009. This large body of articles will be classified according to the following categories: metabolism *versus* transport-based interactions; new substrates, inhibitors and inducers; most represented therapeutic classes and disease entities; largest AUC effects; advances in methodology; noteworthy case reports; challenging and unexplained drug interactions.

10:35 AM – 11:05 AM – BREAK

11:05 AM – 11:15 AM – EXHIBITOR PRESENTATION

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11:15 AM – 11:45 AM - PANEL DISCUSSION

11:45 AM – 1:30 PM – LUNCH

1:30 PM – 1:40 PM – EXHIBITOR PRESENTATION

Cerep

Session II: Enzyme Induction and Inhibition
Chair: Odette Fahmi

A. Enzyme Induction

1:40 PM – 2:15 PM
Overview: Present Status of Enzyme Induction Assays and FDA Requirements *(Jasminder Sahi, Invitrogen Corp. ; Durham ; NC)* The induction of drug clearance pathways, including phase I/II enzymes and transporters, can have important clinical consequences. During the past several years, significant advances have been made in our knowledge of the mechanisms that regulate the expression of genes that determine drug clearance. In vitro human-relevant model systems have been increasingly used to evaluate enzyme induction during preclinical drug development. In this presentation, our current understanding of the mechanisms of enzyme induction and the in vitro methods for assessing the induction potential of new drugs will be discussed in light of the current FDA recommendations.

2 :15 PM – 2:50 PM
Prediction of *In Vivo* Enzyme Induction Data Based on *In Vitro Data* *(Odette Fahmi, Pfizer; Groton, CT)* A major focus of the pharmaceutical industry is directed toward the ability to predict drug-drug interactions such that a high degree of confidence is reached and the potential for over or under prediction is reduced. Recently, numerous methods of predicting CYP3A4 drug-drug interactions have utilized variety
of mathematical models. Comparison of the various approaches used for induction data correlations will be reviewed.

2:50 PM – 3:25 PM
Evaluation of P450 Induction Potential of Mechanism-based Inhibitors – Can We Predict the Outcome? *(Elena Afonina, Sequoia Pharmaceuticals, Inc.; Gaithersburg, MD)* The induction of Cytochrome P450 enzymes (CYP) by xenobiotics can lead to drug-drug interaction and toxicity. CYP induction can occur at both transcriptional and post-transcriptional level. There are several methods to investigate the induction potential of pharmaceutical experimental compounds. Most commonly and widely used assays are PXR based gene reporter assay and CYP activity measurements both allowing high throughput. The assessment of mRNA and protein levels in primary human hepatocytes are considered "gold standard" but are more laborious and time consuming. In our selection process of Pharmacokinetic Enhancers (PKE) that are specific CYP inhibitors, the induction was tested in various assays including PXR gene reporter assay, CYP mRNA and protein measurements. The magnitude and kinetics of induction and effect of mechanism-based CYP inhibitors on different levels of CYP regulation will be discussed.

3:25 PM – 3:55 PM – BREAK

3:55 PM – 4:05 PM - EXHIBITOR PRESENTATION

4:05 PM – 4:40 PM
Evaluation of P450 Induction Using Reporter Cell Lines *(Jerome M. Lasker, Hackensack University Medical Center; Hackensack, NJ)* The enhanced expression of P450 enzymes is often a consequence of exposure to drugs and/or xenobiotics. Such enhanced expression usually stems from the binding of drug ligands to orphan nuclear receptors such as PXR and PPARa, with these ligand-receptor complexes activating transcription of distinct P450 genes. We will address the progress made in using reporter cell lines that stably express nuclear receptors to evaluate a given drug candidate's potential to induce P450 enzymes.

B. Enzyme Inhibition

4:40 PM – 5:15 PM
Use of Pre-pooled Cryopreserved Human Hepatocytes for P450 Inhibition Study *(Albert P. Li, APSciences/IVAL/BRiVAL; Columbia, MD)* In general, liver microsomes prepared from livers of multiple donors are used for the evaluation of enzyme inhibition. Intact human hepatocytes, however, may provide information that is more physiologically relevant than liver microsomes due to the presence of Intact Cell Properties such as intact plasma membranes and uninterrupted, complete, drug metabolizing enzymes and cofactors. To prepare the pre-pooled human hepatocytes, cryopreserved hepatocytes from individual donors are thawed, pooled, and re-cryopreserved. We have developed an optimized procedure for the thawing and re-cryopreservation with no apparent loss of drug metabolizing enzyme activities in the pooled cells after the second thawing. Cryopreserved human hepatocytes pre-pooled from multiple donors can be used for the study as an intact cell equivalent of liver microsomes, providing data representing a "normalized" human population.

5:15 PM – 5:50 PM
The Concept of Pharmacokinetic Enhancement: Benefits of Cytochrome Inhibition *(Sergei Gulnik, Sequoia Pharmaceuticals, Inc.; Gaithersburg, MD)* The oxidation of HIV protease inhibitors (HIV PIs) by cytochrome P450 (CYP) significantly decreases their bioavalability and dictates frequent dosing regimens. To circumvent these problems potent CYP inhibitor, RTV, is commonly used at low doses in today's clinical practice as pharmacokinetic booster of HIV PIs. The strategy of PK boosting with RTV is currently being tested in the development of other antiviral therapies, such as CCR5 receptor antagonists, integrase inhibitors, and HCV protease inhibitors. The inherent activity of RTV against HIV PR may become a significant limitation since administration of sub-therapeutic doses of RTV can lead to the emergence of HIV mutants that are cross-resistant to multiple HIV PIs. The discovery and properties of potent CYP inhibitor with no antiretroviral activity will be presented.

5:50 PM – 6:25 PM
Identification of Reactive Metabolites in Early Drug Discovery *(Phil Butler, Cyprotex Discovery Ltd; Cheshire, United Kingdom)* Reactive metabolites are thought to cause toxicity by covalent modification of cellular macromolecules. Mechanism-based inhibition (MBI) of cytochrome P450, a result of an irreversible interaction between a reactive metabolite and the active site of the enzyme can result in DDI, a major impediment to drug development, and even withdrawal of a drug from the market. Early identification of reactive metabolites in the drug discovery process provides increased flexibility to remove potential liability. Reactive metabolites are most commonly electrophiles that react with nucleophiles such as glutathione (GSH). Traditional reactive metabolite screens focus on simple methods of trapping reactive metabolites with GSH. However, issues relating to sensitivity and selectivity of these methods are common. The current presentation examines the range of experimental strategies available to flag potential reactive metabolite formation and the difficulty in interpreting such data.

6:25 PM – 6:45 PM– PANEL DISCUSSION

END OF DAY 1

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University of Washington Metabolism and Transport Drug Interaction Database

DDI-2009 – DAY 2

Wednesday, May 27, 2009

Session III: Transporters
Chair: Jash Unadkat

8:00 AM – 9:00 AM – REGISTRATION

8:50 AM – 9:00 AM - EXHIBITOR PRESENTATION

Qualyst

9:00 AM – 9:35 AM
Overview: Transporter Drug Interactions: Clinical Relevance *(Jashvant (Jash) D. Unadkat, University of Washington, Seattle, WA)* There is increasing evidence of the involvement of transporters in clinically significant drug-drug interactions. Such interactions can occur at multiple sites including in organs important in drug absorption (e.g. intestine), elimination (e.g. liver and kidneys) and distribution (e.g. brain). To illustrate such interactions, I will focus my presentation on clinically relevant drug interactions at these sites, involving P-glycoprotein, OATPs, and other transporters. IVIVC of these interactions will also be addressed. Supported by NIH MH63641, GM032165, U10HD047892, P50HD044404, and RR00166

9:35 AM – 10:10 AM
Present Status of Transporter Assays in Drug Development *(Jasminder Sahi, CellzDirect/Invitrogen Corp.; Durham, NC)* Membrane transporters contribute to the uptake, secretion and reabsorption of endogenous compounds, xenobiotics and metabolites and are implicated in drug-drug interactions and toxicities. Characterizing transporter mediated interactions of drug candidates helps in more rational design of chemotypes with minimal drug interactions and ideal pharmacokinetic characteristics. This talk will discuss the current status of in vitro xenobiotic transporter assays used to evaluate the renal and hepatic disposition of new chemical entities during drug discovery and development.

10:10 AM – 10:40 AM – BREAK

10:40 AM – 10:50 AM – EXHIBITOR PRESENTATION

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10:50 AM – 11:25 AM
Evaluation of Renal Transporter Mediated Drug-Drug Interaction in Drug Development *(Bo Feng, Pfizer, Inc.; Groton, CT)* Renal uptake transporters located on the basolateral membranes of proximal tubule cells have been shown to play a central role in the renal secretion of a wide range of xenobiotics. Consequently, renal transporter-mediated drug-drug interactions (DDIs) have garnered growing attention in drug discovery and development. Case studies of renal DDI will be discussed in the presentation. Utilizing rat models and in vitro transporter assays, these studies investigated the mechanism responsible for the DDI observed in clinic. As transporter mediated DDI is still an emerging area, these studies have revealed that the in vitro transporter assays are able to help understand the clinical observations, and potentially predict transporter-mediated DDI in clinic.

11:25 AM – 12:00 PM
Transporter-drug Metabolizing Enzymes Cross-talk *(Chris Endres, Amgen Inc.; Seattle, WA)* There is increasing evidence of interplay between drug transporters and drug metabolizing enzymes on pharmacokinetics. For example, the presence and modulation of drug transporter activity can change the extent of drug metabolism and elimination. Likewise, the modulation of drug metabolizing enzyme activity can change the rate or extent of drug transporter dependent processes such as oral absorption, tissue distribution and biliary or renal elimination. This talk will explore the basis of this interplay and provide a mechanistic model to explain and predict these interactions.

12:00 PM – 2:00 PM – LUNCH

2:00 PM – 2:10 PM – EXHIBITOR PRESENTATION

2:10 PM – 2:35 PM
In Vitro Test Systems That Address the FDA's Interest in Drug Efflux Assessment *(Chris Bode, Absorption Systems, Exton, PA)* The US FDA now expects to see *in vitro* data on drug-transporter interactions, from studies carried out in the bidirectional monolayer transport assay format (September 2006 draft guidance on drug interaction studies) because this approach allows a more direct evaluation of transporter-mediated drug transport than other existing model systems. However, all currently available cell lines that are suitable for the specified assay format are, in some sense, non-definitive, as are most currently available pharmacologic probe substrates and inhibitors. Thus, there is an unmet need for more definitive biological test systems for transporter interactions. This presentation will outline the development, characterization and application of CellPort Technologies™, a suite of proprietary human cell lines in which the expression of individual efflux transporters has been targeted, resulting in stable knockdown of one transporter at a time.

2:35 PM - 3:00 PM – PANEL DISCUSSION

Session IV: DDI of Biologics
Chair: Sandhya Girish

3:00 PM – 3:35 PM
PK-based Drug-drug Interactions of Protein Therapeutics: An Industry Perspective *(Frank-Peter Theil, Genentech, Inc.; South San Francisco, CA)* Only few pharmacokinetic-based drug interactions with limited therapeutic relevance have been experienced so far by combining protein therapeutics with other protein and small molecule drugs (SMD). The question remains whether the limited prevalence of those interactions is due to the fact that PK based drug-drug interactions have been underexplored or whether the risk of getting relevant DDIs is lower compared to SMD drug-drug interactions. To answer this question, further clinical investigations as well as mechanistic assessments to understand the potential for DDI of protein therapeutics might be needed. The objectives of this talk will focus on the current mechanistic knowledge of DDIs with protein therapeutics from an industry perspective.

3:35 PM – 4:10 PM
Inhibition of CYP enzymes by Oligonucleotides: Unexpectedly Potent Inhibition of CYP1A2 and CYP2C8 *(David Buckley, XenoTech LLC; Lenexa, KS)* We tested several oligonucleotides as inhibitors of CYP enzymes in human liver microsomes (HLM). Unexpectedly, all of the oligonucleotides tested were surprisingly potent inhibitors of CYP1A2 and/or CYP2C8 with IC_{50} values in the low micromolar range. This presentation will: (1) describe the basic observation of CYP inhibition by oligonucleotides, (2) describe mechanistic follow-up studies including studies of CYP inhibition in human hepatocytes and (3) discuss the possibility that oligonucleotide drugs may have the ability to cause clinically significant inhibition of certain CYP enzymes.

4:10 PM – 4:40 PM – BREAK

4:40 PM – 4:50 AM – EXHIBITOR PRESENTATION

4:50 PM – 5:25 PM
Cytokine Regulation of Cytochromes P450: Consequences for Development and Use of Biologics *(Edward T. Morgan, Emory University; Atlanta, GA)* Inflammation associated with infectious or non-infectious diseases is associated with down-regulation of the expression of cytochrome P450 and other drug metabolizing enzymes. Many of these effects are attributed to proinflammatory cytokines and interferons. This presentation will discuss the cytokines that are involved in P450 regulation in vitro and in vivo, and the issues surrounding the prediction of effects of cytokines and cytokine antagonists on human drug metabolism.

5:25 PM – 6:00 PM
An Overview of Drug-Drug Interaction Potential for Therapeutic Monoclonal Antibodies - A Risk Based Approach *(Sandhya Girish, Genentech, Inc.; South San Francisco, CA)* Therapeutic proteins such as monoclonal antibodies (mAs) are combined with small molecules and/or with other therapeutic proteins. Based on our current knowledge of drug-drug interactions (DDIs) with mAbs and small molecules only modest impact has been demonstrated with respect to pharmacokinetic (PK) DDI. However, there is a need to also address pharmacodynamic (PD) DDI potential. The objectives of this talk will be two-fold: 1) To review the current state of knowledge on the potential for DDI with mAbs and present a few case studies of PK and PD DDI and 2) To provide a risk based approach to assessing potential for DDI for such combinations.

6:00 PM
PANEL DISCUSSION AND CLOSING REMARKS
END OF CONFERENCE

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Pre-Conference	$500.00	$600.00
DDI-2009-Main Conference	$1,250.00	$1,550.00
Combined	$1,600.00	$1,800.00
Exhibitor	$2,000.00	$2,500.00

E.10

Cryopreserved Human Hepatocytes

Stocks of cryopreserved human hepatocytes for suspension use - metabolism applications.

To select your lot, email us at hepaticproducts@invitrogen.com or call one of the numbers below.

Donor Info			Order Info		Cell Biology			Metabolic Activity (pmol/1 x 10^6 cells/min)												
Age	Race	Sex	Catalog number	Lot no.	Cells per vial (millions)	Average viability %	Thawing medium	ECOD	7-HCG	7-HCS	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A[1]	CYP3A[2]	FMO
35	C	F	HMCS2S	Hu0526	4-8	76%	WEM	25.5	396	26.0	39.3	NA	13.5	137	18.2	15.5	NA	356	NA	NA
88	C	M	HMCS1S	Hu0536	4-8	78%	DMEM	41.9	927	69.0	202	NA	38.4	171	70.1	50.7	NA	1,217	NA	NA
62	C	M	HMCS1S	Hu0549	4-8	78%	WEM	30.0	580	35.0	90.1	110	25.7	228	24.9	48.5	176	746	121	872
71	C	F	HMCS2S	Hu0617	4-8	85%	WEM	12.0	1,319	65.0	210	53.0	45.3	257	31.3	78.4	53.4	1,139	234	411
75	C	F	HMCS2S	Hu0619	4-8	88%	DMEM	27.6	1,188	76.0	107	NA	59.0	71.8	43.5	8.40	NA	739	NA	NA
36	C	F	HMCS2S	Hu0624	4-8	76%	WEM	37.0	1,005	78.6	116	68.0	18.2	117	26.0	12.3	45.2	1,096	219	452
79	ME	F	HMCS2S	Hu0749	4-8	82%	WEM	26.3	652	54.4	206	NA	NA	138	29.4	57.5	NA	891	NA	NA
48	AA	F	HMCS2S	Hu0756	4-8	76%	WEM	33.0	498	35.2	124	NA	20.2	119	34.7	42.0	NA	666	NA	NA
49	C	M	HMCS1S	Hu0783	4-8	79%	WEM	23.0	470	25.5	116	NA	NA	193	10.1	22.8	NA	325	NA	NA
66	C	F	HMCS2L	Hu0817	9-12	76%	WEM	31.1	396	35.9	88.6	75.6	13.4	197	17.4	45.8	107	562	144	457
29	C	F	HMCS2L	Hu0823	9-12	80%	WEM	20.8	678	56.4	139	NA	NA	60.4	18.4	48.6	NA	839	NA	NA
83	C	M	HMCS1L	Hu0834	9-12	81%	WEM	16.3	728	96.2	80.1	39.3	NA	109	14.4	20.9	NA	396	78.5	343
38	C	M	HMCS1S	Hu0847	4-8	85%	WEM	18.5	504	38.7	29.3	4.60	NA	67.5	3.79	8.78	NA	408	50.7	NA
57	C	F	HMCS2L	Hu0924	9-12	77%	WEM	47.9	507	11.5	60.7	36.1	9.82	172	55.5	26.4	NA	621	83.1	NA
47	C	F	HMCS2S	Hu0951	4-8	80%	WEM	18.5	949	75.5	37.3	78.9	NA	81.0	1.25	22.4	NA	555	81.2	573
68	C	M	HMCS1S	Hu0957	4-8	72%	WEM	32.6	370	50.2	168	50.2	6.28	212	33.5	56.5	NA	663	95.3	278
27	C	F	HMCS2S	Hu0965	4-8	71%	WEM	23.0	894	55.7	76.1	28.9	NA	397	63.7	19.6	NA	412	79.3	226
68	C	M	HMCS1S	Hu1010	4-8	83%	WEM	15.9	308	36.9	27.0	21.7	7.86	65.3	13.8	56.8	49.3	248	17.5	161
47	C	F	HMCS2L	Hu1015	9-12	87%	WEM	8.58	474	26.3	21.5	20.5	6.13	64.2	47.6	6.02	NA	669	58.5	107
56	C	M	HMCS1S	Hu1029	4-8	80%	WEM	13.6	194	22.3	34.4	15.2	4.08	76.7	15.0	6.26	46.0	551	196	99.1
68	AA	M	HMCS1S	Hu1033	4-8	79%	WEM	24.4	263	78.1	141	22.9	20.8	88.7	20.5	23.4	74.6	450	113	293
65	C	F	HMCS2S	Hu1036	4-8	80%	WEM	52.5	499	27.7	223	71.0	5.57	84.5	7.91	23.2	84.6	1,020	108	200
71	C	F	HMCS2S	Hu1039	4-8	81%	WEM	32.6	383	31.3	39.6	48.9	13.0	94.0	44.7	33.0	70.0	660	75.9	771
42	C	F	HMCS2S	Hu4038	4-8	84%	WEM	14.5	818	30.8	16.7	NA	3.70	63.9	5.00	58.6	NA	439	NA	NA
49	C	F	HMCS2L	Hu4050	9-12	72%	WEM	16.7	790	24.9	83.7	38.1	2.30	40.2	4.90	8.20	39.6	649	54.4	159

F=Female. M = Male. C = Caucasian. H = Hispanic. A = Asian. AA = African American. ME = Middle Eastern. **WEM** = Williams' E Medium. **DMEM** = Dulbecco's Modified Eagle Medium. **CHRM** = Cryopreserved Hepatocytes Recovery Medium. **CYP3A[1]** = Testosterone used as probe substrate. **CYP3A[2]** = Midazolam used as substrate. **Units for Metabolic Activities** = pmol/min/10^6 cells. **NA** = Not available. **TBD** = To be determined; data available soon. **Viability % and Yield** = For thawing either DMEM or WEM was used. Centrifugation conditions were 76 x *g* for 6 min at room temperature (DMEM or WEM). Cells were then resuspended in Incubation Medium (serum-free), and Trypan Blue exclusion was used to determine viability and cell yield. An average viability percentage and cell yield were taken from a minimum of two cell counts. Donor specifications, including serological data, are available upon request. **Viability Stability** = Viability Stability is defined as the percentage of viable cells present after an incubation period of 1 or 2 hours. (Cells are counted in the same manner described for viability % and yield.) **Note:** When CHRM is used as a thawing medium, cell viability and viability stability will typically increase.

—Cryopreserved suspension continued, pg. 2—

—Continued from pg. 1—

Stocks of cryopreserved human hepatocytes for suspension use - metabolism applications.

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Donor Info			Order Info		Cell Biology			Metabolic Activity (pmol/1 x 10^6 cells/min)												
Age	Race	Sex	Catalog number	Lot no.	Cells per vial (millions)	Average viability %	Thawing medium	ECOD	7-HCG	7-HCS	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A[1]	CYP3A[2]	FMO
75	C	M	HMCS1S	Hu4070	4-8	79%	WEM	57.0	1,998	78.0	42.6	NA	11.9	71.2	0.40	70.9	NA	907	NA	NA
59	C	M	HMCS1S	Hu4071	4-8	71%	WEM	64.0	1,413	13.0	46.0	122	3.16	412.0	2.08	19.2	50.8	275	19.3	65.4
48	C	M	HMCS1S	Hu4095	4-8	81%	WEM	21.6	573	48.7	109	29.0	9.70	138.7	12.7	113.8	135	457	86.6	357
26	C	F	HMCS2L	Hu4127	9-12	88%	WEM	32.8	1,550	53.6	101	128	9.12	203	745	21.4	NA	2,300	611	NA
59	C	F	HMCS2S	Hu4140	4-8	77%	WEM	103	917	80.9	22.7	48.3	4.94	74.5	85.0	10.8	759	744	167	117
61	C	F	HMCS2S	Hu4143	4-8	80%	WEM	16.9	144	45.0	10.0	13.4	5.10	132	34.9	10.4	129	358	36.0	253
64	AA	M	HMCS1S	Hu4153	4-8	84%	WEM	50.5	229	51.1	78.5	125	6.85	19.0	2.62	7.88	60.9	280	75.9	81.2
56	C	M	HMCS1S	Hu4159	4-8	72%	WEM	17.6	607	57.9	16.2	130	12.9	80.3	8.58	41.5	NA	479	43.7	134
22	C	M	HMCS1S	Hu4167	4-8	72%	WEM	15.3	1,090	71.5	99.2	304	NA	60.7	38.6	18.7	NA	1,840	471	208
61	C	M	HMCS1S	Hu4171	4-8	83%	WEM	61.2	537	51.8	87.4	201	2.03	144	28.0	21.0	76.3	1,310	248	68.2
31	C	F	HMCS2S	Hu4197	4-8	82%	WEM	21.9	398	27.5	20.5	13.2	1.57	131	4.87	30.8	107	297	17.9	118
58	C	F	HMCS2S	****	4-8	75%	WEM	18.5	194	30.4	86.4	16.2	6.30	84.1	6.48	29.4	117	534	53.7	124
57	C	M	HMCS1L	Hu8008	9-12	78%	DMEM	19.0	1,260	81.2	91.9	15.0	5.42	72.5	6.65	23.1	89.8	582	108	175
60	A	F	HMCS2S	Hu8026	4-8	71%	WEM	30.5	1,118	53.3	17.3	686	66.0	136	8.30	20.5	122	525	63.3	290
25	C	M	HMCS1L	Hu8067	9-12	75%	WEM	9.67	896	64.7	21.0	5.23	NA	35.8	7.45	29.0	NA	189	15.3	304
47	C	M	HMCS1S	Hu8074	4-8	81%	WEM	18.3	236	17.7	30.3	7.01	4.90	130	27.9	14.5	95.0	455	125	148
Poor CYP2D6 metabolizers																				
26	C	F	HMCSPS	Hu0641	4-8	88%	DMEM	40.0	1,589	104	114	NA	4.90	39.5	27.3	0.80	NA	210	NA	NA
62	C	F	HMCSPS	Hu0736A	4-8	74%	WEM	28.6	538	31.2	87.8	26.2	NA	92	18.4	0.30	NA	737	165	309
61	C	M	HMCSPS	Hu0975	4-8	79%	WEM	24.1	386	41.8	50.4	32.5	5.98	551	10.4	2.31	NA	407	33.1	141
52	C	M	HMCSPS	Hu1101	4-8	75%	WEM	10.4	140	20.8	22.1	22.3	5.58	62.5	1.71	0.09	41.3	243	20.8	220
27	C	M	HMCSPS	Hu4022	4-8	69%	WEM	9.85	545	51.8	34.0	16.3	NA	58.3	6.67	0.91	NA	881	172	NA
46	C	M	HMCSPL	Hu4105	9-12	83%	WEM	23.6	309	31.9	111	9.75	5.30	106	18.9	1.05	NA	205	32.6	NA
57	C	F	HMCSPS	Hu4156	4-8	82%	CHRM	60.0	336	17.6	24.3	183	NA	615	2.19	2.09	NA	635	109	132
20	C	F	HMCSPS	Hu8040	4-8	86%	CHRM	54.0	352	21.0	76.9	155	13.4	93.8	19.4	2.45	134	1,598	330	54.6
28	C	F	HMCSPS	Hu8083	4-8	77%	WEM	22.3	291	20.9	18.6	72.3	5.61	116	17.9	1.07	75.6	90.2	14.8	101

F=Female. M = Male. C = Caucasian. H = Hispanic. A = Asian. AA = African American. **ME** = Middle Eastern. **WEM** = Williams' E Medium. **DMEM** = Dulbecco's Modified Eagle Medium. **CHRM** = Cryopreserved Hepatocytes Recovery Medium. **CYP3A[1]** = Testosterone used as probe substrate. **CYP3A[2]** = Midazolam used as substrate. **Units for Metabolic Activities** = pmol/min/10^6 cells. **NA** = Not available. **TBD** = To be determined; data available soon. **Viability % and Yield** = For thawing either DMEM or WEM was used. Centrifugation conditions were 76 x *g* for 6 min at room temperature (DMEM or WEM). Cells were then resuspended in Incubation Medium (serum-free), and Trypan Blue exclusion was used to determine viability and cell yield. An average viability percentage and cell yield were taken from a minimum of two cell counts. Donor specifications, including serological data, are available upon request. **Viability Stability** = Viability Stability is defined as the percentage of viable cells present after an incubation period of 1 or 2 hours. (Cells are counted in the same manner described for viability % and yield.) **Note**: When CHRM is used as a thawing medium, cell viability and viability stability will typically increase.

**** Hu4214, Hu4218, Hu4219 and Hu4220 were produced from the same donor.

Genotyping results

Lot no.	CYP2C9	CYP2C19	CYP2D6	CYP3A5
Hu0526	WT/*3	WT/WT	WT/WT	WT/*3
Hu0536	WT/WT	WT/*2	WT/WT	WT/*3
Hu0549	WT/*3	WT/WT	WT/WT	WT/*3
Hu0617	WT/WT	WT/*2	WT/WT	*3/*3
Hu0619	WT/WT	WT/WT	WT/WT	*3/*3
Hu0624	WT/*2, WT/*3	WT/WT	WT/*4	WT/*3
Hu0685	WT/*3	WT/WT	WT/*4	*3/*3
Hu0749	WT/WT	WT/*2	WT/WT	*3/*3
Hu0756	WT/WT	WT/WT	WT/WT	*3/*3, WT/*6
Hu0783	WT/WT	WT/WT	WT/WT	*3/*3
Hu0817	WT/WT	WT/WT	WT/*4	*3/*3
Hu0823	WT/WT	WT/*2	WT/WT	*3/*3
Hu0834	WT/*2	WT/WT	WT/*4	*3/*3
Hu0847	WT/*2	WT/*2	WT/*3	*3/*3
Hu0924	WT/WT	WT/WT	WT/*4	*3/*3
Hu0951	WT/WT	*2/*2	WT/WT	*3/*3
Hu0957	WT/*2	WT/WT	WT/WT	*3/*3
Hu0965	WT/*2	WT/WT	WT/*6	*3/*3
Hu1010	WT/WT	WT/WT	WT/WT	*3/*3
HU1015	WT/WT	WT/WT	WT/*4	*3/*3
Hu1029	WT/*3	WT/WT	WT/*4	WT/*3
Hu1033	WT/WT	WT/*2	WT/WT	WT/*3
Hu1036	WT/WT	WT/WT	WT/*4	*3/*3
Hu1039	WT/*3	WT/WT	WT/WT	WT/*3
Hu4038	WT/WT	WT/WT	WT/WT	*3/*3
Hu4050	WT/WT	WT/*2	WT/WT	*3/*3
Hu4070	WT/WT	WT/*2	WT/WT	*3/*3
Hu4071	WT/WT	WT/WT	WT/*4	*3/*3

Genotyping results

Lot no.	CYP2C9	CYP2C19	CYP2D6	CYP3A5
Hu4095	WT/WT	WT/WT	WT/WT	*3/*3
Hu4127	*2/*2	WT/WT	WT/WT	*3/*3
Hu4140	TBD	WT/WT	WT/*4	*3/*3
Hu4143	WT/WT	WT/WT	WT/*4	*3/*3
Hu4153	WT/*3	WT/*2	WT/WT	*3/*3
Hu4159	WT/WT	WT/*2	WT/*4	*3/*3
Hu4167	WT/*3	WT/WT	WT/*4	*3/*3
Hu4171	WT/WT	WT/WT	WT/WT	*3/*3
Hu4197	WT/*2	WT/*2	WT/WT	*3/*3
Hu4214	WT/WT	WT/WT	WT/WT	*3/*3
Hu4218	WT/WT	WT/WT	WT/WT	*3/*3
Hu4219	WT/WT	WT/WT	WT/WT	*3/*3
Hu4220	WT/WT	WT/WT	WT/WT	*3/*3
Hu8008	WT/*3	WT/WT	*9/*9	*3/*3
Hu8026	WT/WT	*2/*2	WT/WT	*3/*3
Hu8067	WT/*2, WT/*3	WT/WT	WT/*4	*3/*3
Hu8074	WT/WT	WT/WT	WT/WT	*3/*3
Poor CYP2D6 metabolizers				
Hu0641	WT/WT	WT/WT	*6/*6	*3/*3
Hu0736A	WT/WT	WT/*2	*4/*4	*3/*3
Hu0975	WT/WT	WT/WT	*4/*4	*3/*3
Hu1101	WT/*2	WT/WT	*4/*4	*3/*3
Hu4022	WT/WT	WT/*2	WT/*4	WT/*3
HU4105	WT/*2	WT/WT	*4/*4	*3/*3
Hu4156	WT/WT	WT/WT	*4/*4	*3/*3
Hu8040	WT/*2	WT/WT	*4/*4	*3/*3
Hu8083	WT/WT	WT/WT	*6/*6	*3/*3

Metabolic assay conditions

The commonly tested Phase 1 (P450) and Phase II (UGT, SULT) drug metabolizing enzymes were analyzed for metabolic activity in each of the lots listed above. Cells were carefully thawed in Williams' E Medium (WEM) or Dulbecco's Modified Eagle Medium (DMEM) and resuspended to 1.0 x 10^6 cells/ml in Incubation Medium (serum-free). 0.25 mL or 0.5 mL (ECOD, 7-HC) of cell suspension was aliquoted into a 24-well or a 12-well non-coated plate, respectively, containing the appropriate substrate and placed in a humidified incubator at 37°C, 95% relative humidity, and 5% CO_2 on an orbital shaker. All incubations were performed in triplicate. At the appropriate time point for each substrate, a sample was collected from each well and stored frozen at –70°C until processed by LC/MS/MS or HPLC analysis. Metabolite formation was measured by standard biochemical assays using GLP-validated LC/MS/MS assays (Table 2). At least six calibration standards and 12 quality control samples (at three different concentrations) were used to evaluate the quality of analytical runs.

Table 1—Substrate probes to assess human CYP450 metabolic activity.

Enzyme	Substrate	Concentration	Incubation time	Marker metabolite
CYP1A2	Phenacetin	100 µM	15 min	Acetaminophen
CYP2B6	Bupropion	500 µM	15 min	Hydroxybupropion
CYP2C8	Paclitaxel	20 µM	30 or 45 min	6α-Hydroxypaclitaxel
CYP2C9	Diclofenac	25 µM	15 min	4'-Hydroxydiclofenac
CYP2C19	(S)-Mephenytoin	250 µM	30 min	4'-Hydroxymephenytoin
CYP2D6	Dextromethorphan	15 µM	15 min	Dextrorphan
CYP2E1	Chlorzoxazone	250 µM	15 min	6-Hydroxychlorzoxazone
CYP3A	Testosterone	200 µM	15 min	6β-Hydroxytestosterone
CYP3A	Midazolam	10 µM	10 min	1-hydroxymidazolam
Phase I	7-Ethoxycoumarin	100 µM	30 min	(ECOD) 7-Hydroxycoumarin (7-HC)
Phase II	7-Hydroxycoumarin	100 µM	30 min	7-Hydroxycoumarin Glucuronide (7-HCG) and 7-Hydroxycoumarin Sulfate (7-HCS)
FMO	Benzydamine	250 µM	30 min	Benzydamine-N-Oxide

E.11

Pooled Cryopreserved Human Hepatocytes

Stocks of pooled cryopreserved human hepatocytes for suspension use - metabolism applications.

To select your lot, email us at hepaticproducts@invitrogen.com or call one of the numbers below.

Order Info			Cell Biology			Metabolic Activity (pmol/1 x 10^6 cells/min)												
Catalog no.	Lot no.	No. of donors	Cells per vial (millions)	Average viability %	Thawing medium	ECOD	7-HCG	7-HCS	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A[1]	CYP3A[2]	FMO
HMCS3S	Hu0539-3	2	4-8	84%	DMEM	39.5	751	46.2	50.9	NA	16.5	84.1	22.6	28.7	NA	646	NA	NA
HMCS3S	HuP2001-03	10	4-8	85%	WEM	24.6	221	28.0	118	38.5	14.3	58.5	22.8	16.8	74.8	602	54.7	NA
HMCS3S	HuP53	3	4-8	75%	WEM	44.1	595	48.1	102	49.2	NA	50.3	12.1	9.27	NA	460	NA	216
HMCS3L	HuP61	10	9-12	77%	WEM	24.1	371	35.3	34.8	54.1	11.8	93.0	90.0	18.7	NA	419	149	NA
HMCS3S	HuP88	5	4-8	81%	CHRM	14.7	399	33.3	40.6	55.9	8.20	168	226	46.0	53.0	1,060	167	50.1
HMCS3S	HuP89	10	4-8	75%	WEM	39.2	429	47.5	54.7	40.3	9.90	52.4	22.1	19.5	166	369	115	88.2
HMCS3S	HuP94	10	4-8	79%	WEM	25.6	597	60.0	56.3	38.0	19.6	73.3	150	13.3	77.0	600	500	760

F=Female. M = Male. C = Caucasian. H = Hispanic. A = Asian. AA = African American. ME = Middle Eastern. WEM = Williams' E Medium. DMEM = Dulbecco's Modified Eagle Medium. CHRM = Cryopreserved Hepatocytes Recovery Medium. CYP3A[1] = Testosterone used as probe substrate. CYP3A[2] = Midazolam used as substrate. **Units for Metabolic Activities** = pmol/min/10^6 cells. NA = Not available. **Viability % and Yield** = For thawing either DMEM, WEM, or CHRM was used. Centrifugation conditions were 76 x *g* for 6 min at room temperature (DMEM or WEM) or 100 x *g* for 10 min at room temperature for CHRM. Cells were then resuspended in Incubation Medium (serum-free), and Trypan Blue exclusion was used to determine viability and cell yield. An average viability percentage and cell yield were taken from a minimum of two cell counts. Donor specifications, including serological data, are available upon request. **Note:** When CHRM is used as a thawing medium, cell viability and viability stability will typically increase.

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CYP2C8	Paclitaxel	20 µM	30 or 45 min	6α-Hydroxypaclitaxel
CYP2C9	Diclofenac	25 µM	15 min	4'-Hydroxydiclofenac
CYP2C19	(S)-Mephenytoin	250 µM	30 min	4'-Hydroxymephenytoin
CYP2D6	Dextromethorphan	15 µM	15 min	Dextrorphan
CYP2E1	Chlorzoxazone	250 µM	15 min	6-Hydroxychlorzoxazone
CYP3A	Testosterone	200 µM	15 min	6β-Hydroxytestosterone
CYP3A	Midazolam	10 µM	10 min	1-hydroxymidazolam
Phase I	7-Ethoxycoumarin	100 µM	30 min	(ECOD) 7-Hydroxycoumarin (7-HC)
Phase II	7-Hydroxycoumarin	100 µM	30 min	7-Hydroxycoumarin Glucuronide (7-HCG) and 7-Hydroxycoumarin Sulfate (7-HCS)
FMO	Benzydamine	250 µM	30 min	Benzydamine-N-Oxide